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File: USPT

Nov 30, 1999

DOCUMENT-IDENTIFIER: US 5993661 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Macroporous or microporous filtration membrane, method of preparation and use

Abstract Text (1):

This invention relates to a microporous or macroporous affinity filtration membrane wherein the matrix is composed of chitosan or chitin and the pores are made by dissolution of porogen during the preparation of the membrane. The invention also relates to a method of preparation of the membrane comprising preparing an acidic chitosan solution containing porogen, shaping the suspension into a membrane, and dissolving the porogen by immersing the membrane in an alkaline solution. To prepare chitin membranes, the chitosan membranes are acetylated. The special feature of the membrane is that the pore size can be controlled by varying the size of the porogen. The membranes are suitable for affinity purification of macromolecules.

Brief Summary Text (7):

Recently chitosan membranes have been suggested as affinity membranes for immobilization of various macromolecules having affinity for chitosan. Next to cellulose, chitin (poly (N-acetyl-D-glucosamine)), is the most abundant biopolymer. Chitosan, the deacetylated form of chitin, is soluble in dilute aqueous organic acids but is insoluble in alkaline solutions. Chitosan molecules contain a large number of reactive hydroxyl and amine groups, which can easily attach ligands. In view of its hydrophilicity, excellent film-forming ability, good mechanical properties, and high chemical reactivity (containing hydroxyl and amine groups), chitosan can be an excellent candidate for filtration membranes. Moreover, since chitosan has a positive charge due to the presence of --NH<sub>2</sub> groups, it can be used to selectively adsorb malignant leukemia cells which carry a higher negative charge on their surface than normal cells. Since chitin contains N-acetyl-D-glucosamine units in its structure which can bind certain molecules, it can be employed for affinity purification without further chemical modification. Other advantages of chitosan and chitin are that they are easily available and inexpensive. Moreover, chitin and crosslinked chitosan are insoluble in both acidic and alkaline media making them suitable as filtration membranes.

Brief Summary Text (9):

Currently, there is no suitable method available for the preparation of microporous or macroporous chitosan membranes wherein the size of the pores can be controlled. The most common method to prepare microporous chitosan membranes is the phase-inversion process, using a large molecular weight organic compound as a porogen. The process involves three steps: (1) casting of a solution of the membrane containing a porogen and partial evaporation of the solvent; (2) sol-gel transformation and generation of pores via the addition of a solvent for the porogen; and (3) heat treatment for stabilizing the pore structure and improving the mechanical properties. This method requires rigorous control of various parameters, particularly the kind and amount of porogen and evaporation conditions (time, humidity and temperature). Generally, the porogens employed in the phase-inversion methods for preparing hydrophobic membranes were organic compounds of low molecular weight such as acetone, dimethyl formamide, dimethyl sulfoxide, benzene, etc. To obtain large pores in chitosan membranes, the relatively large molecule of poly(ethylene glycol), molecular weight 35,000, was used as porogen (Zeng and Ruckenstein, 1996 J. Membr. Sci. vol 117:271-278). Although relatively high

permeability membranes were obtained, their mechanical properties were not satisfactory, and they had to be placed on another support.

Brief Summary Text (10):

So far, microporous or macroporous chitin membranes have not been available, primarily because no suitable solvent and porogen could be found. A few solvents, such as the mixtures trichloroacetic acid-chloral hydrate-dichloromethane (Brine and Austin, 1975 ACS Symposium Series, Church T. D., Eds., American Chemical Soc., vol 18, p505), dimethylacetamide (DMAC)-LiCl (Rutherford and Austin, 1977 Proc. of the First International Conf. on Chitin and Chitosan, Muzzaralli, R. A. A., Priser, E. R., Eds., MIT Sea Grant Program, Cambridge), and N-methyl-2-pyrrolidone-DMAC-LiCl (Uragami et al., 1981 Polym., vol 30:1155-1156) have been tried. However, it was either almost impossible to completely dissolve chitin in these solvents, or required a long time, followed frequently by degradation.

Brief Summary Text (12):

An object of the present invention is to provide a macroporous or microporous filtration membrane for affinity purification of macromolecules, wherein the matrix comprises chitosan or its acetylated form, chitin, and the size of the pores can be controlled.

Brief Summary Text (13):

Another object of the present invention is to provide a method for the preparation of chitosan or chitin membranes wherein the matrix of the membrane is formed around a porogen of desired size. The porogen particles are then dissolved to form the membrane pores.

Brief Summary Text (14):

A further object of the invention is to provide a method for affinity purification of molecules having an affinity for chitosan or chitin.

Drawing Description Text (6):

FIG. 3 is a schematic diagram of a cartridge used to house chitosan or chitin membranes for affinity filtration.

Drawing Description Text (7):

FIG. 4 is a graph illustrating the adsorption of lysozyme on the chitin membrane cartridge at 20.degree. C.

Drawing Description Text (9):

FIG. 6 is a graph illustrating HPLC elution profiles of (a)--pure ovalbumin; (b)--pure lysozyme; and (c)--lysozyme separated from egg white using a chitin cartridge.

Detailed Description Text (5):

The present invention is concerned with a porous affinity membrane wherein the matrix comprises chitosan or chitin, and wherein the pores are created by dissolution of the porogen. The pores can be in the micro--(diameter 0.1 .mu.m to 1.0 .mu.m) or macro--(diameter greater than 1.0 .mu.m) range depending upon the size of the porogen selected for the preparation of the membranes.

Detailed Description Text (8):

In one illustrative embodiment, the porogen is silica. Since silica is available in several sizes, a wide range of pore size can be achieved. In a more preferred embodiment, the size of silica particles is from about 15 .mu.m to about 40 .mu.m which results in an average pore size of 19.8 .mu.m for chitosan membranes and 17.9 .mu.m for chitin membranes.

Detailed Description Text (17):

Chitin microporous or macroporous membranes are obtained via acetylation of the corresponding chitosan membranes with acetic anhydride in methanol. The N-acetylated chitosan (chitin) membrane has a stronger chemical resistance than chitosan membrane, being insoluble in 5 vol % aqueous solution of acetic acid (pH 2.5) and in 5 wt % aqueous NaOH solution. This increased chemical resistance is most likely due to the presence of COCH.sub.3 group, which decreases the elongation upon increasing

the extent of crystallinity.

Detailed Description Text (18):

The method of the present invention can also be employed to prepare composite membranes, in which chitosan is blended with synthetic polymers such as, but not limited to, polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), polyethylene oxide (PEO), poly amides, polyacrylamides, polymethacrylate and polyhydroxyethyl methacrylate, or natural polymers such as, but not limited to, gelatin, collagen, dextran, agarose, silk, cellulose and cellulose derivatives. Such combinations would be useful in improving the properties of polymer membranes, such as blood compatibility, mechanical properties and biodegradability.

Detailed Description Text (19):

The present invention is not limited to flat sheets, but can be used in other forms including, but not limited to, hollow fibers and beads. Flat membranes can be housed in a support assembly. In a preferred embodiment, the support assembly is a plate type filtration cartridge wherein, multiple membranes can be stacked. Stacking of multiple membranes increases the adsorptive capacity of the membranes.

Detailed Description Text (20):

In contrast to most commercial membranes, the membranes of the present invention contain a large number of active groups (--OH and/or NH.sub.2). Therefore the membranes of the present invention can be used, without any further amplification of the number of active groups, in various applications such as affinity membranes. Since chitin membranes contain N-acetyl-D-glucosamine units in its structure, it can be used to bind macromolecules that have an affinity for this group. Such macromolecules include, but are not limited to, lysozyme and wheat germ agglutinin.

Detailed Description Text (21):

Chitosan membranes can bind molecules that have an affinity for glucosamine. Such molecules include, but are not limited to, protein A and Cibacron.TM. Blue F3GA dye. Other potential uses for macroporous and microporous chitosan and chitin membranes include, but are not limited to, agents for wound dressing, hemostatic bandages, metal chelating agents, enzyme carriers, agents for cell immobilization, and blood filters to remove selected cells.

Detailed Description Text (31):

Conversion of Macroporous Chitosan Membranes to Macroporous Chitin Membranes

Detailed Description Text (32):

To prepare chitin membranes, the corresponding chitosan membranes were acetylated after removal of the alkaline solution used to dissolve the porogen particles. The acetylation of chitosan membranes to chitin was carried out via its immersion into a stirred solution of 100 ml methanol containing 5 ml of acetic anhydride for 1 hour at 50.degree. C. The membranes were then removed from the solution and washed successively with methanol and distilled water, followed by treatment of the membrane with 5 wt % aqueous NaOH solution overnight to remove the CH.sub.2 OH acetylated groups. Finally, a white macroporous chitin membrane was obtained after washing with distilled water until neutral conditions. Table 2 presents a comparison of the chemical resistance and mechanical properties, and Table 3 presents a comparison of the physical properties of chitin and chitosan membranes. The mechanical properties of the chitosan and chitin membranes were determined at 20.degree. C. using an Instron.TM. universal testing instrument (Model 1000). The gauge length was 20 mm and the extension rate 10 mm/min. The specific adsorption areas of chitosan and chitin macroporous membranes were determined by the BET (Brunauer-Emmett-Teller) method using a Micromeritics.TM. ASAP 2000 instrument. The porosities of the chitosan and chitin membranes were obtained by determining their swelling in water and using the following expression:

Detailed Description Text (35):

Morphology of Chitosan and Chitin Membranes:

Detailed Description Text (36):

Scanning electron microscopy was employed to investigate the morphology of the chitosan and chitin membranes. The specimen were prepared as follows: the wet

membrane was wiped with a filter paper to remove the excess water present on the surface of the membrane, then framed on a petri dish to prevent shrinkage along the surface, and allowed to dry. The membranes were fractured under liquid nitrogen and the fractured surfaces were coated with a thin layer of carbon before scanning. FIGS. 1(a), 1(b) and 1(c) show that the pores are distributed uniformly indicating that the silica particles were dispersed uniformly.

Detailed Description Text (41):

Preparation of Composite Membranes containing Chitosan and Synthetic Polymers

Detailed Description Text (42):

In one embodiment of the invention, chitosan is blended with synthetic polymers to make composite membranes. Chitosan and PEO were dissolved individually in 1 vol % aqueous acetic acid solution containing 10 wt % glycerol. Then the two solutions were mixed in various proportions. The silica particles (15-40 .mu.m) were suspended via stirring in the mixture; this was followed by casting the suspension on a rimmed glass plate. After drying at room temperature, the dried membrane was immersed in 5% NaOH solution at 80.degree. C. for 2 hours, followed by washing with distilled water. This produced a macroporous chitosan-poly(ethylene oxide) blend membrane.

Detailed Description Text (45):

Preparation of Composite Membranes containing Chitosan and Natural Polymers

Detailed Description Text (46):

In one embodiment of the invention, composite membranes containing chitosan and collagen or chitosan and gelatin were prepared. One wt % solutions of chitosan and collagen or gelatin were prepared by dissolving them individually in a 2 vol % aqueous acetic acid solution. The individual solutions were then mixed in a 1:1 volume ratio. Silica particles (15-40 .mu.m) were added with vigorous mixing, followed by casting the suspension on a rimmed glass plate and drying at room temperature. The dried membrane was immersed in 5% NaOH solution at 60.degree. C. for 5 hours, followed by washing with distilled water.

Detailed Description Text (52):

In one embodiment of this invention, the membrane is in the form of a hollow fiber. For preparing hollow fibers, an acidic chitosan solution containing silica particles of desired size is placed into a cylinder and extruded with a piston through a spinneret into a coagulation bath (aqueous 5% wt NaOH, which may contain ethanol or methanol). While not intending to be bound by any particular theory, it is believed that this solidifies the fiber by deprotonating the amine group. This is followed by drawing the fiber through a washing bath (deionized water) to remove the sodium hydroxide, and then through an acetone bath to dehydrate the fiber. Glycerine may be added into the spin dope, the coagulating solution and washing bath to prevent rupture during drying. Subsequently the fiber is immersed in a NaOH solution at 80.degree. C. to dissolve the silica particles and to generate the porous fiber. A hollow fiber spinneret can be employed to prepare chitosan hollow fiber. Treatment with a cross-linker, as in Example 2, is needed to stabilize the membranes. Micro- or macroporous chitin fibers or hollow membranes can be prepared by acetylating the chitosan fiber or hollow fiber with acetic anhydride.

Detailed Description Text (55):

In another embodiment of this invention, chitosan and chitin beads are prepared. To prepare chitosan beads, a suspension of silica particles in acidic chitosan solution is dropped through a nozzle, using compressed air, into a stirred NaOH or NaOH-methanol solution to form chitosan beads. The formed beads are filtered and washed with deionized water and methanol, followed by drying. The silica-containing beads are immersed in a 5 wt % NaOH to dissolve the silica particle, and to generate micro- or macroporous chitosan beads. A cross-linker, for example, glutaraldehyde, hexamethylene diisocyanate or ethylene glycol diglycidyl ether, can be used to harden the beads.

Detailed Description Text (56):

For the preparation of beads, these cross-linkers are preferred over epichlorohydrin since the use of these cross-linkers results in hard beads that are easier to process than the epichlorohydrin treated beads. To prepare chitin beads, the

chitosan beads can be treated with acetic anhydride.

Detailed Description Text (59):

In one embodiment of the invention, the membrane is used for affinity purification of lysozyme which has a known affinity for the D-glucosamine moieties of chitin. A 1 mg/ml solution of lysozyme in 0.1 M phosphate buffer (pH 8.0) containing 1 M NaCl was prepared and loaded at a flow rate of 1, 5, or 15 ml/min into the chitin cartridge of Example 8. The ratio of the concentration of lysozyme in the effluent (C) and the initial concentration of lysozyme (C.sub.o) is plotted as a function of time in FIG. 4. The time required to achieve saturation was about 20 min for 15 ml/min (c; curve 6), about 30 min for 5 ml/min (b; curve 5) and more than 70 min for 1 ml/min (a; curve 4). The adsorption was followed by washing with phosphate buffer for 10 min at a flow rate of 15 ml/min, and by elution with 0.1 M acetic acid solution at 1, 5, and 15 ml/min, until no protein was detected in the effluent. The effluent was collected and the concentration determined spectrophotometrically. The elution profile is presented in FIG. 5. About 40, 10 and 5 minutes were needed for the elution flows of 1 ml/min (a; curve 7), 5 ml/min (b; curve 8), and 15 ml/min (c; curve 9) respectively to remove 95% of the strongly bound protein.

Detailed Description Text (62):

In one embodiment of the invention, the membrane was used for separation of lysozyme from egg white. Hen egg white was first separated from fresh eggs. Then 10 ml of homogenized egg white was diluted with 90 ml of 0.1 M phosphate solution (pH 8.0) containing 1 M NaCl, followed by filtration and centrifugation at 100 g for 20 min. Finally, 65 ml of supernatant was pumped through the chitin cartridge of Example 8, at 1 ml/min, followed by 10 min washing at 15 ml/min and about 15 min elution at 5 ml/min. The purity of the lysozyme was examined by High Performance Liquid Chromatography (HPLC) using a wide-pore CBx HPLC column, 5 .mu.m, 7.75 mm.times.100 mm. The flow rate was 1 ml/min, the mobile phase A binding buffer was 25 mM MES ((2-N-morpholino) ethanesulfonic acid), pH 5.6 and the mobile phase B eluting buffer was 1 M NaOAc, pH 7.0. The sample size was 100 .mu.l. The various profiles in FIG. 6 represent pure ovalbumin (a; curve 10), pure lysozyme (b; curve 11) and lysozyme from egg white (c; curve 12). The purity of lysozyme from egg white was estimated to be higher than 98% and its specific activity was 54,003 units/mg protein.

Detailed Description Paragraph Table (2):

TABLE 2	chem resistance in mechanical
properties (dry/wet) elongation 5 wt % 5 vol % tensile strength, at membrane NaOH solution HOAc solution MPa break,	% chitosan
insoluble soluble 7.37/0.90 6.1/102.2 <u>chitin</u> insoluble insoluble 9.23/1.09 4.6/29.3	

Detailed Description Paragraph Table (3):

TABLE 3	flux (ml/min/ cm.sup.2) at spec
average pressure thickness, porosity, adsorption pore size drop of membrane .mu.m % area m.sup.2 /g .mu.m 3/5 psi	chitosan 119
75.2 1.8 19.8 17.6/30.8 <u>chitin</u> 132 62.2 1.6 17.9 15.0/28.9	

Current US Cross Reference Classification (4):

210/500.29

Other Reference Publication (7):

C.J. Brine and P.R. Austin, (1975), Renatured chitin fibrils, films and filaments, ACS Symposium series vol. 18, pp. 505-518.

Other Reference Publication (8):

F.A. Rutherford and P.A. Austin, (1977), Marine Chitin Properties and Solvents, Proc. First International Conference on Chitin and Chitosan, pp. 182-192.

CLAIMS:

11. A method for the preparation of a membrane according to claim 10, comprising the steps of:

- a. suspending porogen particles in an acidic solution comprising chitosan
  - b. shaping the suspension into a membrane;
  - c. extracting the porogen by contacting the membrane with an aqueous alkaline solution;
  - d. removing the alkaline solution; and
  - e. converting chitosan to chitin by treating the membrane with acetic anhydride.
39. The method of claim 38, wherein the membrane is housed in a support assembly.
40. The method of claim 39, wherein the support assembly is a plate type filtration cartridge.



☐ 3. Document ID: US 5505890 A

L9: Entry 3 of 10

File: USPT

Apr 9, 1996

US-PAT-NO: 5505890

DOCUMENT-IDENTIFIER: US 5505890 A

**\*\* See image for Certificate of Correction \*\***

TITLE: Process for manufacturing cellulose acetate membranes

DATE-ISSUED: April 9, 1996

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Duweg; Gustav	Wuppertal			DE
Steinfeld; Lothar	Schwelm			DE
Ansorge; Wolfgang	Essen			DE

US-CL-CURRENT: 264/177.14; 210/500.23, 210/500.29, 210/500.3, 264/178R, 264/186,  
264/187, 264/191, 264/195, 264/200

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
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☐ 4. Document ID: US 5505859 A

L9: Entry 4 of 10

File: USPT

Apr 9, 1996

US-PAT-NO: 5505859

DOCUMENT-IDENTIFIER: US 5505859 A

TITLE: Hollow fiber for dialysis and process of manufacturing

DATE-ISSUED: April 9, 1996

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dunweg; Gustav	Wuppertal			DE
Breidohr; Hans G.	Wuppertal			DE
Baurmeister; Ulrich	Wuppertal			DE
Tilgner; Hans G.	Mulheim am Rhein			DE
Stein; Uwe	Wuppertal			DE

US-CL-CURRENT: 210/500.23; 210/490, 210/500.29, 210/500.35, 210/500.36, 264/178R,  
264/199, 264/200, 96/6

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
Draw Desc	Image									

☐ 5. Document ID: US 5427684 A

L9: Entry 5 of 10

File: USPT

Jun 27, 1995



US-PAT-NO: 5427684  
DOCUMENT-IDENTIFIER: US 5427684 A

TITLE: Dialysis membrane composed of polysaccharide ether II

DATE-ISSUED: June 27, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Diamantoglou; Michael	Erlenbach			DE
Dunweg; Gustav	Wuppertal			DE
Rintelen; Thomas	Schwelm			DE

US-CL-CURRENT: 210/500.23; 210/500.27, 210/500.29, 264/199, 264/41, 264/563, 536/20

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
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☐ 6. Document ID: US 5171444 A

L9: Entry 6 of 10

File: USPT

Dec 15, 1992

US-PAT-NO: 5171444  
DOCUMENT-IDENTIFIER: US 5171444 A  
**\*\* See image for Certificate of Correction \*\***

TITLE: Dialysis membrane made of polysaccharide ether

DATE-ISSUED: December 15, 1992

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Diamantoglou; Michael	Erlenbach			DE
Lemke; Horst-Dieter	Obernburg			DE

US-CL-CURRENT: 210/500.23; 210/500.29

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments	KMC
Draw Desc	Image									

☐ 7. Document ID: US 5087366 A

L9: Entry 7 of 10

File: USPT

Feb 11, 1992

US-PAT-NO: 5087366  
DOCUMENT-IDENTIFIER: US 5087366 A  
**\*\* See image for Certificate of Correction \*\***

TITLE: Biocompatible dialysis membrane comprising a mixed polysaccharide ester

DATE-ISSUED: February 11, 1992

INVENTOR-INFORMATION:

US-CL-CURRENT: 210/500.23; 210/500.29, 210/500.38

8. Document ID: US 4962140 A

Oct 9, 1990

DOCUMENT-IDENTIFIER: US 4962140 A

DATE-ISSUED: October 9, 1990

## INVENTOR- INFORMATION:

US-CL-CURRENT: 524/35; 210/500.29, 422/129

☐ 9. Document ID: US 4872983 A

Oct 10, 1989

DOCUMENT-IDENTIFIER: US 4872983 A

**\*\* See image for Certificate of Correction \*\***

DATE-ISSUED: October 10, 1989

## INVENTOR- INFORMATION:

US-CL-CURRENT: 210/500.29; 210/321.71

[illegible]

☐ 10. Document ID: US 4714555 A

L9: Entry 10 of 10

File: USPT

Dec 22, 1987

US-PAT-NO: 4714555

DOCUMENT-IDENTIFIER: US 4714555 A

TITLE: Agent for separation

DATE-ISSUED: December 22, 1987

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Shibata; Tohru	Himeji			JP
Okamoto; Ichiro	Himeji			JP

US-CL-CURRENT: 210/635; 210/198.2, 210/198.3, 210/500.29, 210/644, 210/656, 210/658, 502/404

Full	Title	Citation	Front	Review	Classification	Date	Reference	Sequences	Attachments
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